SOFIE; An Optimized Approach for Exposure Tests and Sediment Assays

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Abstract – The use of biological sensors, rather than chemical measurements, appears promising for risk assessments of contaminated environments provided that the assay is able to mimic natural conditions. As an alternative to the standard bioassay protocol, a new technique was developed that meets this requirement, leaving sample and geochemical conditions in tact. Exposure tests were conducted with two aquatic species that occur in sediment and water, respectively. Comparison between the two methods showed that the standard protocol tends to overestimate risks for PAHs, and underestimates the risks for heavy metals, in terms of accumulated amounts. Sample handling largely affected chemical speciation, and exposure concentrations deviated from the ones observed in the undisturbed setting. This new approach may contribute to better-founded quality criteria for sediments.

1. Introduction

Regulatory quality standards are primarily developed to indicate, scale or rank the environmental risks of contaminants. For soils and sediments, most countries legally prescribe the chemical extraction of total amounts of contaminants as a first step for risk assessment. Contaminants have mostly been sequestered over a long period of time and may only become available to organisms to a limited extend. The introduction of the bioassay, as a second step, generally increased the understanding of the concept of chemical versus biological availability. This is illustrated in Figure 1. Aquatic oligochaetes that have been exposed to a number of sediments with a varying degree of cadmium content appear to be particularly susceptible to dissolved fractions. Although location L1 is ranked as seriously contaminated, based on total extracted amounts of cadmium from sediment, *Limnodrilus* spp. accumulated most Cd at the "clean" location L2.
The thought that chemical speciation generally determines the adverse toxic effects in biota is well accepted (reviewed by, among others, Campbell in1995). In recent years, some techniques have become available to actually measure the availability (i.e., labile metal species) in "natural" solutions. These techniques make use of the (combined) electrochemical, diffusive, or competitive properties of metal ions. Table 1 summarizes some of the most promising techniques. However, only few of these may find their use in exposure tests because of their disturbance (or destruction) of the sample, or the inability to measure speciation under anoxic conditions.

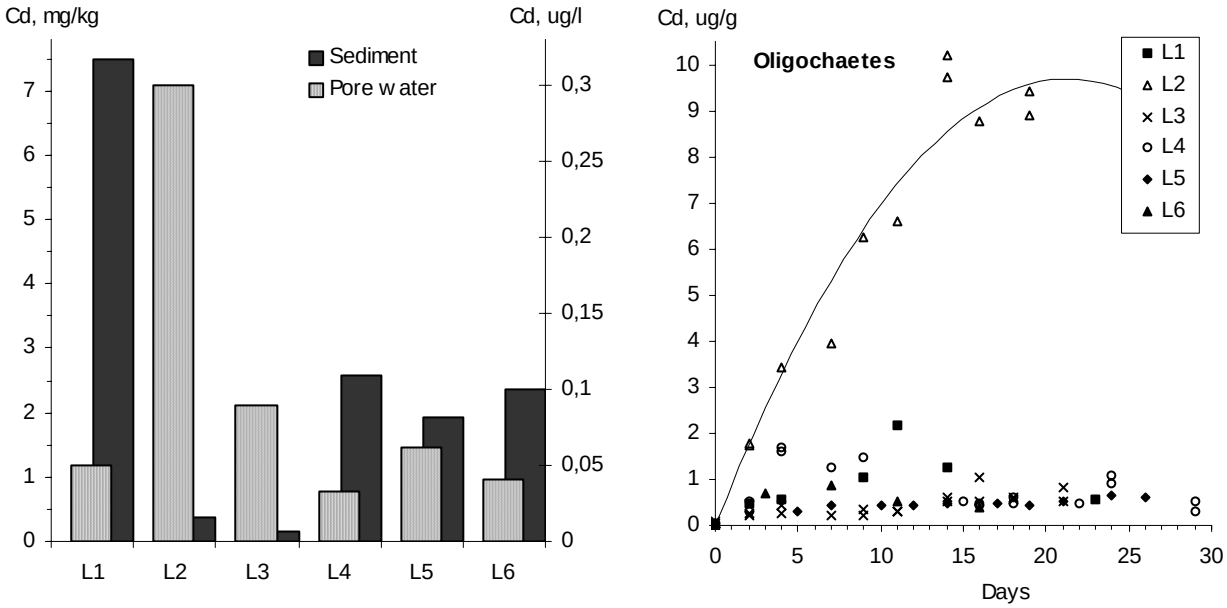
The purpose of bioassays is typically to determine the toxicity of ambient waters and sediments. Some assays focus on the determination of the exact concentration at which a chemical becomes toxic to an organism, so a biological response is used as a sensor. In order to be useful, this response should be repeatable. Ideally, bioassays are used to make predictions of environmental toxicity required for contaminant management (Chapman, 1989; Blum and Speece, 1990) and should therefore at all times aim at extrapolating results to the field. Sediment bioassays are a relatively new approach to determine the environmental effects of sequestered metals in sediments. This type of assay has been discussed widely (e.g., Schwartz, 1987; Luoma, 1995) in terms of limitations and advantages. Sediments typically consist of oxidized layers, overlying reduced ones. It is well known and documented how redox processes affect metal chemistry (e.g., Aller, 1978; Vink, 2002) and consequently the outcome of the test. Nevertheless, sediments are generally collected as bulk samples that contain this initial redox zonation. Mostly, these samples are further manipulated by sieving, mixing, and oxygenation in order to increase homogeneity and repeatability. Indeed, variability is likely to be increased if sediments are not homogenized after collection, but these types of handling may destroy important reaction zones that are typical for the sediment in question and its environmental effect.
Data from standardized bioassays (i.e., protocols) generally provide the clear-cut answers that managers find useful as the basis for decisions, regardless of the accuracy to natural conditions. Therefore, these data continue to be the principal tool in the development of regulatory quality standards (Luoma, 1995). If a bioassay is conducted in order to meet the objective of field-extrapolation, i.e., to assess the environmental risks under natural conditions, a method of minimal sample manipulation should be considered.

Recently, a new approach was introduced that allows for a non-destructive handling and true-to-nature assessment of water/sediment systems. This approach is the "sediment or fauna incubation experiment", or SOFIE, and was developed to measure chemical speciation of metals over redox zones in undisturbed systems, while simultaneously conducting a single or multiple species bioassay. In this chapter, we discuss the performance of this approach and compare it with the outcome of a standardized protocol for sediment or surface water bioassays. Tests were conducted with the sediment-dweller *Chironomus riparius* (mosquito larva) and *Daphnia magna* (water flee) for a range of contaminants. Since this study aims at the straightforward comparison between the outcome of both methods, the speciation measurements conducted with SOFIE are not reported here.

TABLE 1. Operational metal speciation sensors.

Method		Chemical separation	Measured species	Response time	Remark	Ref
AdSV	Adsorptive stripping voltammetry	Electrochemical	Free metal + labile species	Minutes	Complex detection	Kaldova & Koopanica, 1989
CLE	Competing ligand exchange	Exchange reaction with ligands	Free metal (+ very labile species?)	Seconds	Critical modification and handling	Apte & Batley, 1995
DET	Diffusive equilibrium in thin film	Porous gel	Free metal + penetrable complexes	Days	Ionic strength limitations	Davison e.a., 1991
DGT	Diffusive gradient in thin film	Porous gel	Free metal + labile penetrable complexes	Days	Critical gel composition	Zhang & Davison, 1995
DMT	Donnan membrane technique	Charged pore membrane	Free metal + part of cationic penetrable complexes	Days	Critical pore size	Lampert, 1982
GIME	Gel impregnated microelectrode	Porous gel + electrochemical	Free metal + labile penetrable complexes	Minutes	Surface/lability interaction	Tercier & Buffle, 1996
ISE	Ion selective electrode	Liquid partition without countercharge transport	Free metal	Seconds	Interference from other metals and organic matter	Berner, 1963
PLM	Permeation liquid membrane	Liquid partition with countercharge transport	Free metal + labile complexes	Minutes	Diffusion-controlling step	Buffle e.a, 2000

FIGURE 1. The availability-concept in practice: cadmium in sediment, pore water, and oligochaetes at various locations (L1-6).



2. Methods of exposure

2.1 The standard bioassay

The widely applied protocol for water and sediment testing for single species is what we call here the "standard bioassay". This protocol is based on the TRIAD-guidelines of 1993 and describes the general procedure of the test, including handling of samples and test species. From this protocol, two test procedures were followed:

I. Standard test with sediment-dwellers

For tests using *Chironomus riparius* as a test organism, use is made of a water/sediment system. A bulk sample of the top 10 cm of a sediment is collected from the field and mechanically homogenized. The sample is then mixed 1:4 v/v with DSW ("standard water"; demineralized water with mineral additives). The water/sediment mixture is shaken for 24 hours at ambient atmosphere. 100 ml of the suspension is decanted in dishes to settle, after which test species are introduced. After 28 days of exposure, the organisms are collected by sieving, allowed to void their gut content for 24 hours, and freeze dried.

II. Standard test with water-dwellers

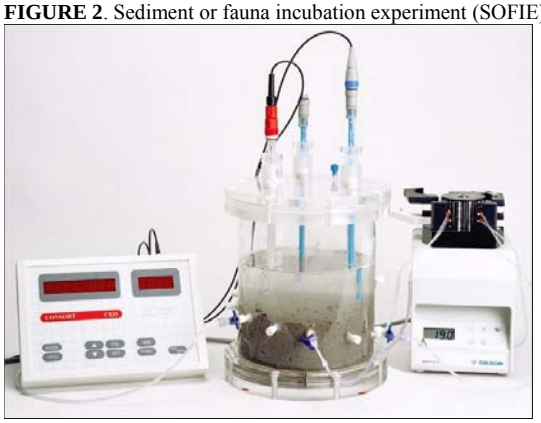
For tests using *Daphnia magna*, the protocol prescribes the use of pore water, which is separated from the homogenized sediment by centrifugation (2500 g, 30 min) and stored in bottles. Exposure is carried out in 100 ml glass beakers and pore water is refreshed twice a week. Individuals are periodically collected, rinsed with Elendt-medium and analyzed for metals (not PAHs because of mass and detection limitations).

2.2 The sediment or fauna incubation experiment (SOFIE)

This study was performed with a novel experimental technique (EU-patent nrs. 1018200 / 02077121.8, October 2001, J. Vink, Rijkswaterstaat), which was introduced as Sediment Or Fauna Incubation Experiment, in short SOFIE. This device or "cell" is shown in Figure 2.

SOFIE is based on the competing ligand exchange technique, which is integrated in a (pore)water probe and combined with a bioassay setting. Full and detailed experimental possibilities have been described earlier (Vink, 2002) and in another chapter. The cell consists of a circular core, which is used as a sampling device to obtain undisturbed water/sediment systems. Field samples are taken including the overlying surface water in such a way that the physical and geochemical integrity of the sample (e.g., bulk density, redox status) is guaranteed. After sampling, the core is closed at the bottom with a shutter plate and is mounted on a base socket.

FIGURE 2. Sediment or fauna incubation experiment (SOFIE).



The sample does not leave the body core but is now part of the cell. A top socket is attached, ensuring a gas-tight fitting with the body core with an internal silicone seal. The top plate of this socket has four gas tight connectors for any commercial electrode to probe sediment or overlying water.For experimental scenario purposes, aerobic or anaerobic conditions in the water-sediment system may be manipulated by flushing the atmospheric head space with any desirable gas. The cell wall contains fifteen gas tight connectors for probes. These probes (diameter 1mm, 50 mm length) consist of a semi-permeable polyethersulfon which acts as a membrane to discriminate colloidal fractions > 0.1 µm. This mesh size is impermeable to bacteria, so pore water that passes the polymer is sterile. The probes can be directly coupled onto micro ion-exchange columns (MICs), as was described in detail by Vink (2002), to separate free metal ions from other forms of organic and inorganic metal ligands in solution at the reigning geochemical status of the probed sediment layer.

For a simple, straightforward comparison between the standard bioassay and SOFIE, sediment samples were collected from the river Meuse, The Netherlands and analyzed for a large amount of properties. An undisturbed water/sediment interface was taken with the cell's body core at 0.4 m water depth. After steady state metal concentrations were determined, 125 individuals of *Chironomus riparius* were introduced into the SOFIE cell and sampled at the same time intervals as in the standard bioassay. Tests with *Daphnia magna* were performed in the same way.

2.3 The presence of food during exposure

In an additional test with *Daphnia magna,* the effect of feeding during exposure was investigated. This test was performed in a two-compartment SOFIE cell, which has two separate chambers that divide the sample while it is cored. In both compartments, daphnids were introduced in comparable amounts as in the standard bioassay. Daphnids in compartment A were fed with the alga *Chlorella,* their natural food source, while those in compartment B were not fed. Organisms and water samples were collected from the cell compartments on several occasions.
Metal concentrations in chironomids and daphnids were determined by digestion in 500 µl of 14.9 M HNO_3 (Ultrex) at 180°C in a microwave. The digest was analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, PerkinElmer Elan 6000). Polycyclic Aromatic Hydrocarbons (PAHs) in chironomids were determined by refluxing for 6 hours with hexane. Analyses were done with an Agilent HP-Liquid chromatograph using a fluorescence detector and a Vydac 201TP54 reverse phase C18 column. Dolt-2 (certified by the Community Bureau of Reference, BCR, Brussels, Belgium) was used as biological reference material.

3 Results and discussion

The sediment composed of 14% <2µm, 2.3 % organic C, 0.55 inorganic C, pH=7.2, and was moderately contaminated with metals (As: 8.5 mg/kg dw; Cd: 1.57; Cr: 27.7; Cu: 25.7; Hg: 0.21; Ni: 20.8; Pb: 79; Zn: 321) and various PAHs (ranging from 100-800 ng/g dw). Collection of pore water from the sediment by homogenization and centrifugation, according to the protocol, generally increased metal concentrations, most significantly for Pb and Cd. Table 2 shows the concentrations that were measured at the start of the exposure test in the standard bioassay and in SOFIE's surface water.

TABLE 2. Pore water concentrations (µg/l, sd) after sample handling (standard bioassay) and in sediment of SOFIE.

	Standard bioassay		SOFIE	
Zn	23.3	(4.7)	28.1	(5.6)
Cu	6.5	(0.7)	2.7	(0.2)
Pb	17.8	(3.6)	1.3	(0.3)
Ni	6.4	(0.7)	2.0	(0.2)
Cr	4.2	(0.3)	2.4	(0.2)
Cd	0.26	(0.01)	0.03	(0.002)

FIGURE 3. PAH (top) and heavy metals (bottom) in chironomids after 28 days. Error bars denote the variation in analytical recovery.

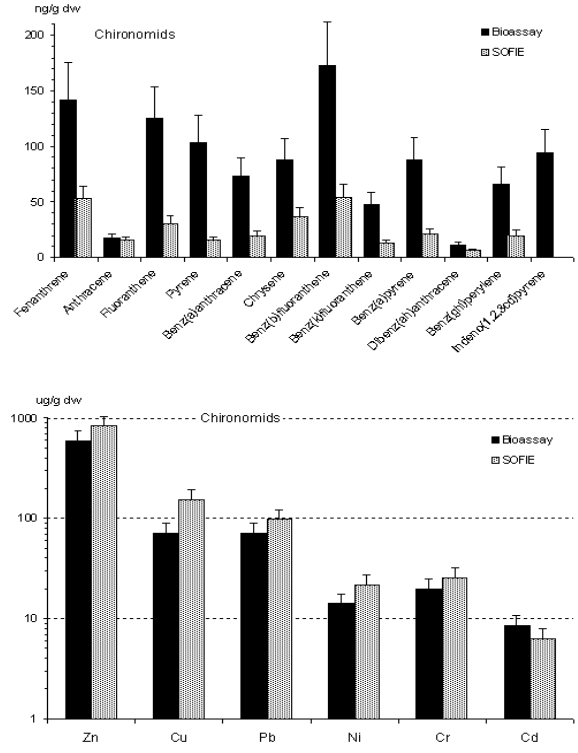
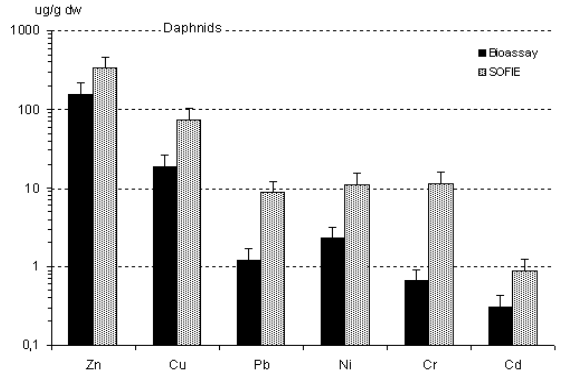


FIGURE 4. Metals in daphnids after 16 days of exposure.



The collected time data for chironomids are, for reasons of survey ability, summarized in Figure 3 which shows body concentrations of polycyclic aromatic hydrocarbons (PAH) and heavy metals after four weeks of exposure. The data quite clearly show that in the standard bioassay, chironomids take up PAHs in significant larger amounts than in the SOFIE environment. Although we did not perform or aimed at a mechanistic study here, it is likely to assume that sampling handling (and in particular stirring and homogenization) has liberated PAHs from the organic sediment matrix by unlocking and exposing organic surface areas. Many authors have described this phenomenon.

Bioaccumulated metals are shown in Figure 3 and 4. Accumulated amounts for both test species are lower in the standard bioassay than those collected from SOFIE (note the log-scale, differences are significant). This is valid for all metals. Although this observation may appear somewhat counterintuitive (oxidation leads to liberalization of metals from sulfides and should therefore increase the (bio)available fraction of metals), the explanation is quite straightforward. Indeed, oxidation leads to enhanced metal concentrations, as is shown in Table 3. Following chemical thermodynamics, this primarily yields large free ion activities. Simultaneously, iron and manganese are oxidized from its reduced state into highly reactive oxyhydroxides (either precipitated or as colloids). Free metal ions have a high affinity for these newly formed sorption sites and are quickly adsorbed and immobilized. In the case of the standard Daphnia bioassay, where sediment is absent, there is obviously no delivery from the sediment (e.g., by diffusion and desorption) to the aqueous phase. Daphnids take up metals from solution, and eventually face declining exposure concentrations to a point where concentration levels may become inhibiting to uptake. Figure 5 shows copper for an example. This trend was observed for all metals in the standard bioassays, but not in the SOFIE cells where concentrations remained relatively constant during the exposure period or showed some release from the sediment.

Figure 6 shows the effect of feeding daphnids during exposure. The effect of adding the alga *Chlorella* is profound (note the log-scale). In most cases, bioaccumulated amounts of metals are reduced some orders of magnitude compared to the non-feeding test. Since alga are always present in surface waters in varying extents, it is obvious that this test is closer to exposure conditions as they occur in the field.

Results show that dissolved metal concentrations change during exposure. These time varying changes may follow first order reaction kinetics, i.e., dC/dt = kC, so the external concentration, or exposure concentration, at a given time C(t), is written as:

$$C(t) = C_i e^{k_0 t}$$

In which C_i is the initial exposure concentration, and k_0 is rate term describing the increase or decrease of the initial concentration during the test. A time/concentration-dynamic, two-compartment model approximated metal bioaccumulation patterns of both test species. Body concentrations vary in time, and relate to uptake from the water phase at a certain rate. At the same time, uptake is accompanied by elimination, which is kinetically directed by the organism itself.

FIGURE 5. Copper concentrations in overlying water at time of sampling (SA) and during exposure (days) in the standard bioassay (■) and SOFIE (□) for chironomids (top) and daphnids (bottom). Q1, Q2 are body concentrations from the standard bioassay and SOFIE, respectively. While concentrations in the undisturbed water/sediment system remain relatively constant or increase slightly, those in the standard bioassay show large variation due to the handling and manipulation of the sample, followed by exhaustion from solution.

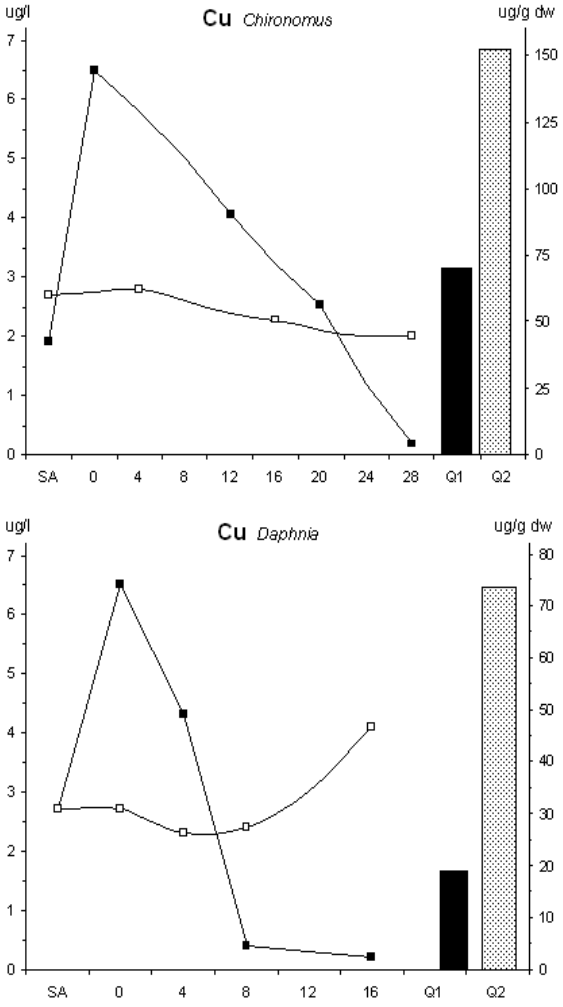
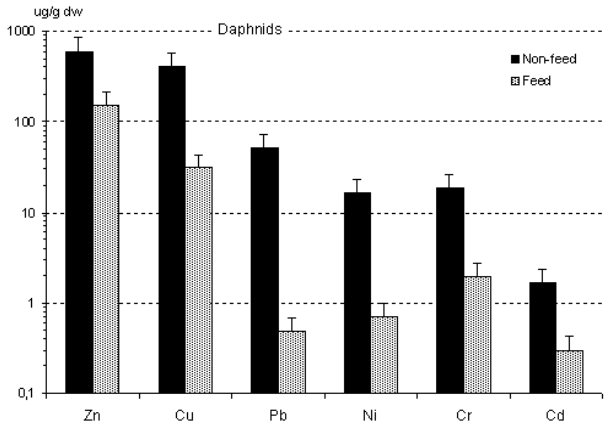


FIGURE 6. Heavy metals in *Daphnia magna* in two feeding scenarios.



Hence, one may write:
$dQ/dt = k_1 C(t) - k_2 Q(t)$,
with Q being the internal body concentration ($\mu g.g^{-1}$), k_1 and k_2 are uptake and elimination rate constants, respectively (day^{-1}), and t is time. This yields, for Q(0)=0:

$$Q(t) = \frac{k_1 C_0}{k_2 - k_0}(e^{-k_0 t} - e^{-k_2 t})$$

With the introduction of k_0, time dependent concentrations determine the overall exposure of organisms, and thus Q(t).
Table 3 summarizes the results of the uptake model. In the standard bioassay, initial concentrations differ between chironomids and daphnids. This is the result of sampling handling and the absence of sediment in the daphnid test. From the viewpoint that bioassays should deliver generic, true-to-nature outcomes, this appears an undesirable starting point. Although *Chironomus* is a sediment dweller and *Daphnia* is not, both species encounter contaminant exposure in an environment that consists of both sediment and surface water where bioavailability is a combined action of the two compartments.
The rate by which exposure concentrations change during the tests is represented by k_0. Positive values denote an increase, negative values a decrease in concentrations. The outcome clearly shows that in the standard bioassay, k_0-values are in all cases negative, and larger than those observed in the assays that were performed with SOFIE. The effect is most profound in the daphnid tests where k_0-values diverge both in direction as in magnitude. For all metals, and both test species, the two-compartment model performed better with the data that were acquired from the SOFIE set-up.

TABLE 3. Initial concentrations C_i (µg/l) in the exposure medium, model parameters k_0, k_1, k_2 (1/day) and correlation coefficient.

	Standard bioassay						SOFIE					
	Zn	Cu	Pb	Ni	Cr	Cd	Zn	Cu	Pb	Ni	Cr	Cd
Chironomids												
C_i	9.2	3.7	3.8	2.8	2.0	0.08	45.8	2.7	2.5	4.9	1.0	0.39
k_0	-0.040	-0.11	-0.120	-0.030	-0.050	-0.100	-0.007	-0.015	-0.018	0.018	0.045	0.001
k_1	44.05	7.41	5.96	18.67	9.56	10.19	1.55	4.53	47.53	7.52	2.04	3.39
k_2	1.15	6.51	9.71	8.84	3.72	1.07	0.09	0.11	1.98	1.61	0.04	0.08
r^2	0.80	0.38	0.54	0.68	0.44	0.14	0.90	0.79	0.96	0.55	0.67	0.53
Daphnids												
C_i	23.3	6.5	17.8	6.4	4.2	0.26	45.8	2.7	2.5	4.9	1.0	0.39
k_0	-0.114	-0.258	-0.350	-0.215	-0.151	-0.272	-0.040	0.047	0.101	0.023	-0.062	0.106
k_1	10.54	0.94	0.005	0.03	0.26	0.31	0.66	1.44	0.01	0.01	0.11	0.07
k_2	8.31	12.94	12.72	2.24	12.80	12.67	0.11	-0.06	-0.35	-0.32	-0.17	0.01
r^2	0.92	0.13	0.71	0.89	0.58	0.47	0.97	0.99	1.00	1.00	0.99	0.85

4. Conclusions

Based on the outcome of water/sediment testing of two aquatic species with the standard protocol and with the sediment or fauna incubation experiment, SOFIE, the following conclusions are drawn:

- Sample handling, such as homogenization and oxygenation, affects the chemical speciation of the exposure medium for both organic as inorganic contaminants. Disadvantages of this manipulation (disrupting chemical speciation and bioavailability) outweigh the advantages (homogenization to increase reproducibility) by far, and should therefore seriously be reconsidered or applied with great caution.
- Tests conducted with the standard protocol tend to *overestimate* uptake of PAHs by chironomids, and *underestimate* uptake of heavy metals by chironomids and daphnids. This may generate both false-positive and false-negative results, respectively.
- Aquatic bioassays should be conducted in sediment/water systems, regardless whether the tested species are sediment-dwellers or not. The physical, chemical, and biological interaction between sediment and the water phase is a site-specific characteristic that determines the ultimate risk of contaminants.
- SOFIE provides the necessary tool to conduct risk assessments in a close-to-nature setting. It may therefore contribute to establish better-founded quality criteria for natural waters.

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